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EGGS: CONVENIENTLY PACKAGED ANTIBODIES. METHODS FOR PURIFICATION OF YOLK IgG

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Eggs from immunized chickens may provide a convenient and inexpensive source of antibodies. We describe two simple and efficient methods for purification of IgG from yolk. The antibody is rendered useful for most currently employed immunological techniques. Amounts of antibody corresponding to almost half a litre of antiserum may be recovered from a chicken in one month.

INTRODUCTION

Immunology has benefited from the easy dissection of the chicken immune system by bursectomy (Glick, 1956), and the phylogenetic distance between birds and mammals which have made the chicken the antibody producer of choice when cross-reacting antibodies against mammalian antigens are desired. A hitherto unrecognized advantage may lie in the observation that the hen, like her mammalian counterparts, provides her young with antibodies as protection against hostile invaders (Brambell, 1970). The immunoglobulin (IgG) content of the yolk is actually higher than that of the hen's serum (Rose et al., 1974). Apparently this has not been generally realized among immunologists, or it may be that difficulties in separating the IgG from the profusion of yolk lipids has impeded the exploitation of this readily available source of antibody, which may amount to the equivalent of more than 100 ml of serum per week. Antibodies have become a central tool in the biological sciences and we believe that we need not emphasize the difference in skill or work required between bleeding rabbits and collecting eggs.

A number of methods for separation of proteins (levitins) from lipoproteins (lipovitellins) and the rest of the yolk lipids have been described (Schmittle, 1950; Martin et al., 1957; Martin and Cook, 1958; Parkinson, 1972). It may be recalled that almost 50% of the yolk is non-aqueous material. We have found the published methods of limited value for routine processing of yolk as they involve extraction with organic solvents with rather

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low yields of antibody, or prolonged high speed centrifugation.

We therefore sought to develop simple methods to circumvent the troublesome tendency of the IgG to separate out together with the yolk lipids. The two methods reported below appear sufficiently simple and efficient to warrant consideration of hens as antibody producers irrespective of the inherent advantages of chicken antibodies.

METHODS AND RESULTS

The first step in method I (see Fig. 1) is a modificiation of the dextran sulphate precipitation method of separating lipoproteins from serum (Burstein and Praverman, 1957). The entire procedure is carried out at room

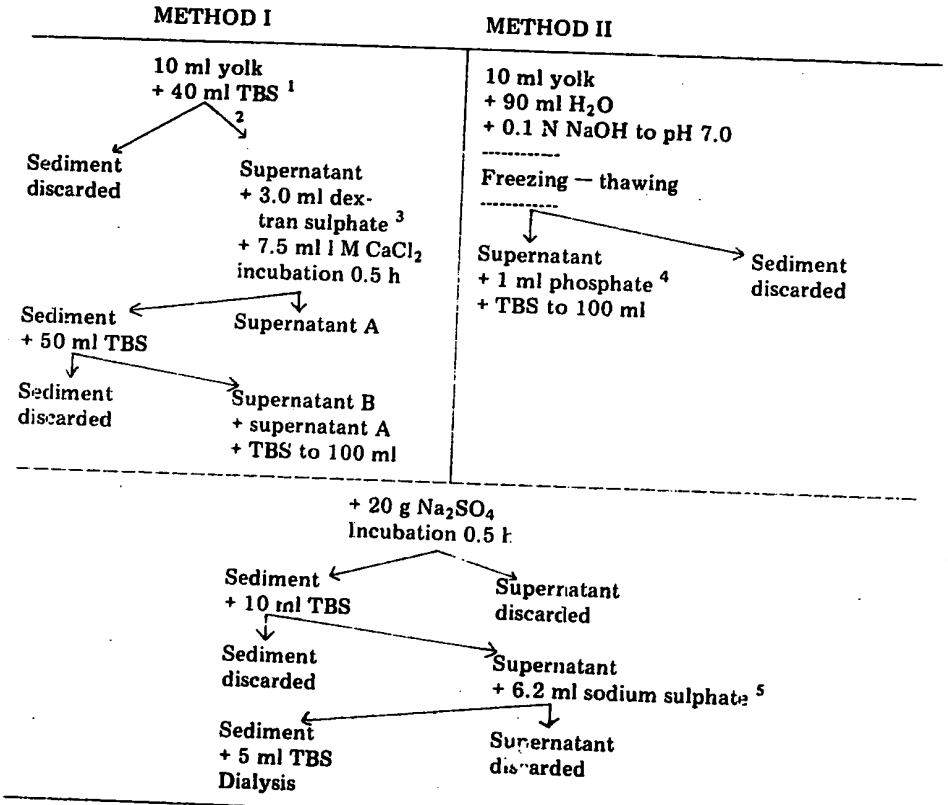

Fig. 1. Purification of yolk IgG. [1] TBS: Tris hydroxymethylaminomethane-buffered saline: 0.14 M NaCl, 0.01 M Tris/HCl, pH 7.4, 0.1% NaN_3. [2] Centrifugation at 2 000 x *g*, 20 min room temperature. [3] 10% (w/v) dextran sulphate (Pharmacia) in TBS. [4] 0.4 M phosphate, pH 7.6. [5] 36% (w/v) anhydrous sodium sulphate (the volume added is corrected for the volume and sodium sulphate content of an average precipitate).

temperature. The yolk is separated from the white, and the yolk membrane cut open. The yolk is poured out and diluted with Tris-buffered saline (TBS). The diluted yolk may be stored for prolonged periods in the cold. Precipitate formed on dilution and during storage may be separated by centrifugation, allowing a reduction of the amount of dextran sulphate necessary for the next step. Dextran sulphate is mixed with the diluted yolk after which calcium chloride is added to induce precipitation of excess dextran sulphate. The sediment is washed with TBS to extract protein carried with it, and the two supernatants pooled. (The first supernatant will be cloudy and subsequent salt precipitation impeded if too little dextran sulphate and calcium chloride is added. To remedy this simply add more of the reagents, mix and repeat incubation and centrifugation.) The IgG in the supernatants may now be purified and concentrated by sodium sulphate precipitation. Cooling must be avoided in this step as this will induce crystallization of the sodium sulphate. The precipitate is dissolved in a convenient volume of TBS and the IgG is ready for use after dialysis against the buffer of choice (e.g. TBS) and removal of insoluble material by centrifugation. Alternatively, if an IgG preparation of greater purity is desired, this may easily be obtained by reprecipitation of the IgG with 14% sodium sulphate (Benedict, 1967), by addition of a suitable volume of supersaturated sodium sulphate (28% or 36% w/v) with stirring. We routinely attain a recovery of 70—80% of the total yolk IgG in the first sodium sulphate precipitate, corresponding to 10—15 mg of IgG per ml of yolk, only little of which is lost during further purification. In the first precipitation step 2 M ammonium sulphate may be used instead of sodium sulphate (18.5 g ammonium sulphate is added per 100 ml). Recoveries were estimated by adding ^{125}I-labelled chicken IgG (Jensenius, 1976), by titration with ^{125}I-labelled rabbit L chain of antibody from the eggs of chickens immunized with rabbit IgG (Jensenius, 1976) and by rocket immunoelectrophoresis against rabbit anti-chicken L chain antiserum (Crone et al., 1972).

Method II uses the aggregation of yolk lipid at low ionic strength and neutral pH. We found that the IgG segregated together with the lipid at pH values lower then 7.0. The yolk is diluted with 9 vol of water and frozen and thawed after adjusting the pH to 7.0. Enough of the lipids may then be removed by centrifugation to make possible precipitation of the IgG by sodium sulphate or ammonium sulphate. The yield of IgG is somewhat lower (50—70%) than by method I but method II is nevertheless considered a useful and cheap alternative.

IgG constituted more than 90% of the protein in preparations made by both methods, as judged from photometric scanning of analytical SDS polyacrylamide gel electrophoresis (Fig. 2).

We have immunized chickens with rabbit IgG or IgA by conventional adjuvant methods and found the yolk to contain roughly as much antibody as the serum although the appearance of antibody in the yolk was somewhat delayed in agreement with results obtained by other procedures (Patterson

et al., 1962; Orlans, 1967). The yolk antibody is presumably mainly of the IgG class as IgM and IgA cannot be demonstrated in the yolk (Rose et al., 1974). Only small amounts of antibody were found in the egg white.

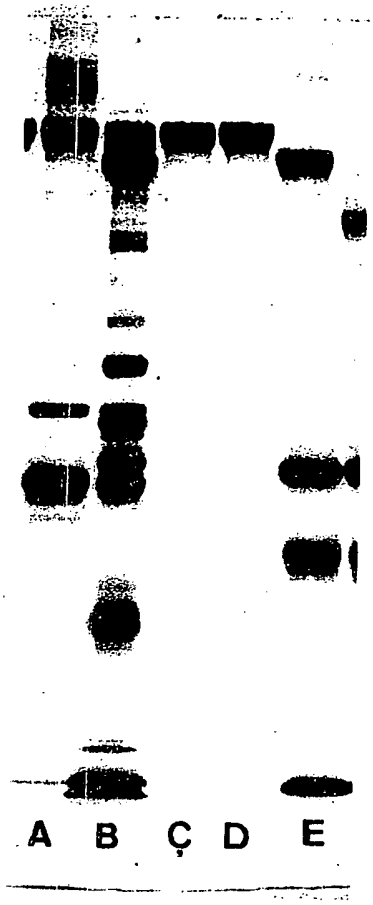

Fig. 2. SDS polyacrylamide gel electrophoresis on a 5—15% gradient gel of (A) 1 μl chicken serum; (B) 1 μl yolk (5 μl of yolk diluted with 4 vol of TBS and centrifuged); (C) 10 μg of yolk IgG purified according to method I; (D) chicken serum IgG purified by sodium sulphate precipitation, DEAE ion exchange chromatography and gel permeation chromatography; (E) molecular weight marker mixture composed of human IgG, bovine serum albumin, egg albumin and cytochrome C.

DISCUSSION

Chicken antibodies may be used in variety of procedures. We have used them in radioimmunoassays as first or second antibodies (unpublished)

and in immun electrophoretic techniques (Hau et al., 1980). In certain cases it may be advantageous to enhance precipitation by including 4% (w/v) of polyethylene glycol (PEG 6 000) in the buffer system used. The cross-reactivity of chicken antibodies may be considered an advantage or a nuisance depending on the use to which they are put. It should, however, be realized that (a) the production of cross-reacting antibodies cannot be taken for granted; (b) they may be manifest in some applications, i.e., binding techniques or gel precipitation but absent in others such as radioimmunoassays (unpublished observations with chicken anti-rabbit IgG and IgA antibodies); and (c) all the standard methods for removing unwanted antibodies apply also to chicken antibodies. It should be realized that chicken IgG does not fix mammalian complement (mixed complement is needed for cytolysis; Benson et al., 1961), does not bind *Staphylococcus aureus* protein A (Kronvall et al., 1970), and seemingly does not bind to mammalian Fc receptors. Yolk antibodies are stable in situ in the cold for long periods (Branly et al., 1946).

We believe that eggs will turn out to be a convenient source of antibodies for many purposes. One may even speculate that, by providing large amounts of neatly wrapped antibody, eggs from suitable immunized chickens might be a useful and harmless therapy for some intestinal infections, if steps can be taken to minimize the degradation of the antibody be intestinal proteolytic enzymes. This would be analogous to the idea of treating various infections with milk from immunized animals (Campbell and Petersen, 1964) which has a much smaller IgG content than yolk.

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